Message

From: Strynar, Mark [/O=EXCHANGELABS/OU=EXCHANGE ADMINISTRATIVE GROUP (FYDIBOHF23SPDLT)/CN=RECIPIENTS/CN=5A9910D5B38E471497BD875FD329A20A-STRYNAR, MARK]
Sent: 2/26/2020 12:43:26 PM
To: Johnsie Lang [jrlang@ncsu.edu]; Lindstrom, Andrew [Lindstrom.Andrew@epa.gov]; Chernoff, Neil [Chernoff.Neil@epa.gov]; Hill, Donna [Hill.Donna@epa.gov]; Huang, Hwa [huang.hwa@epa.gov]
Subject: RE: Decision on Manuscript ID ez-2019-006809

Still a bad decision. I think we repackage it immediately and send it off to another journal that may be receptive to this tox study. Maybe Tox Sci?

Mark

From: Johnsie Lang <jrlang@ncsu.edu>
Sent: Wednesday, February 26, 2020 4:04 AM
To: Strynar, Mark <Strynar.Mark@epa.gov>; Lindstrom, Andrew <Lindstrom.Andrew@epa.gov>; Chernoff, Neil <Chernoff.Neil@epa.gov>; Hill, Donna <Hill.Donna@epa.gov>; Huang, Hwa <huang.hwa@epa.gov>
Subject: Fwd: Decision on Manuscript ID ez-2019-006809

---------- Forwarded message ---------
From: **Environmental Science & Technology Letters** <onbehalfof@manuscriptcentral.com>
Date: Tue, Feb 25, 2020 at 11:51 PM
Subject: Decision on Manuscript ID ez-2019-006809
To: <jrlang@ncsu.edu>

25-Feb-2020

Journal: Environmental Science & Technology Letters
Manuscript ID: ez-2019-006809
Title: "Toxicity of Balb-C Mice Exposed to 1,1,2,2-Tetrafluoro-2-[1,1,1,2,3,3-hexafluoro-3-(1,1,2,2-tetrafluoroethoxy)propan-2-yl]oxyethane-1-sulfonic acid (PFESA-BP2)"
Author(s): Lang, Johnsie; Strynar, Mark; Lindstrom, Andrew; Farthing, Amy; Huang, Hwa; Schmid, Judith; Hill, Donna; Chernoff, Neil

Dear Dr. Lang:

Thank you for submitting your manuscript for publication in Environmental Science & Technology Letters.

Per your request, we sent your manuscript out again for review and the reviewers again indicated that the work is not appropriate for ES&T Letters.

The fundamental concern here is that the manuscript does not communicate sufficient new information for the broad Environmental Science & Technology Letters audience. Given the comments of the reviewers, you will need to consider submission of this work elsewhere.

I hope that the reviewers' comments are of help to you if you choose to revise the manuscript for submission to another

journal.

Sincerely,

Dr. Daniel Schlenk
Associate Editor
Environmental Science & Technology Letters
Email: schlenk-office@estlett.acs.org
Phone: 951-827-2018
Fax: 202-354-4613

Reviewer(s)' Comments to Author:

Reviewer: 3

Comments:
In this report, toxicological studies were carried out for PFESA-BP2, a byproduct of Nafion polymer that has been detected in surface water, drinking water and human sera. After oral exposure to PFESA-BP2, the concentration in mice serum was detected, and liver toxicity was evaluated by histopathological observation and clinical chemistry measurement. Overall, the results support the conclusion that PFESA-BP2 exerts liver toxicity to mice and is potentially harmful to humans. Hepatotoxicity has been commonly found for many other PFAS including PFOS and PFOA. Given the close similarity in chemical structure between PFESA-BP2 and PFOS, the reported hepatotoxicity is not surprising and quite predictable.

The authors need to justify the importance of their finding by directly comparing the data of PFESA-BP2 and PFOS regarding their environmental occurrence, human exposure and toxicity. Based on a few limited reports, PFESA-BP2 levels in surface water and drinking water are still relatively low and less than PFOS. This is also the case for human exposure level. Regarding hepatotoxicity on mice, how do the histopathological and clinical chemistry results of PFESA-BP2 look like when compared with those of PFOS at the same dose levels? And how would it look like if the comparison is made at the same or similar serum concentrations?

Please also provide the reason for the selection of the doses used in the experiment, and LD50 value of PFESA-BP2 if it is available.

Reviewer: 4

Comments:
In this manuscript (ez-2019-006809) the authors present findings of a study to describe toxicological effects of "PFESA Byproduct 2" (BP2) in Balb-C mice orally exposed for seven days to a range of doses (0 - 6 mg/kg). This is a novel compound in the class of per- and polyfluoroalkyl substances (PFAS) found in the Cape Fear River and associated with a fluorproduction facility. To date, there are not toxicological data associated with this PFAS. The study was conducted to evaluate serum and liver concentrations and liver-associated biomarkers. Comments, questions, and suggestions are included for the authors' consideration.

Comments, questions, and suggestions.
Overall
1. Throughout the manuscript there are some grammatical errors/subject-verb confusions. It is recommended that a technical editor/writer be consulted to clarify these confusions. A few have been pointed out in the review comments.
2. Throughout the manuscript the authors missed several opportunities to provide additional details especially with respect to experimental design considerations. It is suggested that these details be added as this manuscript could have

potential importance for decision-makers.

Abstract
3. Line 24. Suggest changing "exists" to "exist" as data are plural.

4. Lines 24-25. It is suggested that in the Abstract, the authors include a brief rationale statement about how they selected their doses and exposure duration.

5. Line 28. The authors may wish to clarify that the doubling in liver size is in weight/mass and not just appearance.

6. Line 30. It is suggested that the authors include a parenthetical to highlight the serum markers of liver injury that were affected.

7. Lines 30-33. It is suggested that the authors clarify what they mean by "percent" of BP2. The indicate that it is the percent in serum relative to the amount administered, but this is not typically how these values are expressed. Similar comment with respect to liver accumulation. Some clarification is needed.

Introduction
8. Line 38. Are there national regulations for some PFAS in the U.S. or do the authors mean national regulations in other countries? It is suggested that the authors clarify.

9. Lines 44-45. Is the compound under study in the submitted manuscript known to exist as a by-product of manufacturing Nafion polymer or is this assumed? Who has established that it is a known by-product?

10. Lines 45-47. Could the authors please clarify what types of PFAS require pre-manufacture notice under TSCA.

11. Line 50. If the compound under study in the submitted manuscript a DuPont by-product or a Chemours by-product? Didn't DuPont spin off all fluorocompound manufacture into Chemours? The authors may want to clarify this.

12. Lines 52-55. This entire paragraph seems overly generalized. What do the authors mean by "traditional" wastewater treatment processes? What processes are/were employed by Chemours? Are the authors certain that lack of sufficient treatment resulted in the discharge of many different PFAS into water sources? What about direct (allowable or not) discharges? Atmospheric deposition? The authors are encouraged to remove this paragraph or provide more details.

13. Lines 58-62. The authors have confused subjects in this paragraph. As it currently is written, it reads that the US EPA used a non-targeted analytical method to estimate BP2 concentrations in a report to the NC DEQ. It is suggested that the authors modify the sentence to read: "In a September...the Unites States Environmental Protection Agency (USEPA) described how they used a non-targeted..."

14. Lines 79-81. The authors may wish to clarify that the widespread presence of BP2 in human serum samples was from a very small sample of human study participants from the region.

Methods
15. Could the authors please indicate the choice for Balb-c mice. One sentence would be enough.

16. Lines 106-108. Could the authors please provide a rationale for the seven-day dosing period, the chosen dose ranges, and why there was such a disparity in sample sizes among the dose groups?

17. Line 179. The Statistical Evaluation indicates that variables were analyzed by a two-way ANOVA. Concentration of BP2 administered is one independent variable. If variables were analyzed separately by sex then what was the other independent variable? Could the authors please indicate the two independent variables used in the two-way ANOVA.

Results

18. Throughout. Could the authors please indicate percent or fold difference among endpoints rather than just indicating "significantly?" Significantly can mean biological or statistical significance and the authors do not indicate which. Percent or fold-change difference would provide some context for readers.

19. Lines 188-189. Could the authors please indicate somewhere in the Methods how often body weights were collected (daily during the exposure period?) and what overt behavioral observations were collected and how often?

SI
20. Figure S1. Please indicate, with arrows, areas of histopathological change.

Reviewer: 5

Comments:
The authors are addressing an important topic on a novel PFAS contaminant - characterizing health effects of Nafion BP2 and how they are related to internal dose is important information. Because of that, there are more details needed in this manuscript. The study is not well defined, as your block design is not mentioned in the methods, the statistics did not examine for a block effect, and it is not clear that the study was performed without bias. I hope that these comments will help solidify the methods.
1. Line 32 - I suggest that this is phrased differently - instead of dose-dependence, maybe "was not altered by dose"
2. somewhere in the introduction, possibly near lines 46-49, you should say in plain language that by-product release into the environment doesn't follow the same laws as those commerce items that go through TSCA, therefore no health information requirement.
3. line 55 - discharge of PFAS
4. line 100 - state whose IACUC (US EPA?)
5. In the experimental design section, describe the block design used in this study that is referred to elsewhere in the paper. How many animals per block, which treatments were in each block, and how did you test for block effect? Additionally, how were the animals assigned to the groups? Were the groups weight equalized prior to treatment (you didn't state that there was a weight assessment prior to exposures, yet they were weight based)? How were the doses assigned to groups and did you do anything to assure lack of bias? Ex., line 119 no mention of weights prior to dosing.
6. The way the methods are written, the collection of serum is non-traditional. Did you collect the blood once the animals were CO2 anesthetized or after exsanguination (as written) and if you really did spin at this speed and that length of time, you likely lysed cells and changed the content from traditional serum prep methods.
7. There are some oddities about the liver collection also. What portion (lobe) of the liver was collected? line 124 says "one portion". That is not acceptable as not all portions are equal. Was the same portion collected for every animal? Section 2.3 needs clarification: were any control samples sectioned and stained, where is Pathogenesis LLC located, how were the single samples from this large study chosen? Was the liver size known when choosing samples for sectioning? There is no way to know if this is representative of the groups and the n is unacceptable. I am suggesting an n=3, at least. Also, please add original magnification to the Supp Figure 1, as the bars are unreadable. The "control" section does not appear "normal" - did the pathologist compare to historical controls at all?
8. Was clinical chemistry performed in singles or duplicates? How much volume was used? Where did the standards come from?
9. Mass spec details - line 160, what were the standards range and LOQ for Nafion BP2? Line 161 - were analyzed without dilution (instead of directly), as I am guessing that formic acid and ACN were added to the controls also before running them. Line 165 - extracted how? Do you mean that 10% of all samples had 2 extractions as defined above or that 10% of the sample volumes were extracted twice (how)? This sentence needs clarification. Lines 174-6 doesn't make any sense - when did you need to measure the level of PFOA in this study?
10. As the stats section was the first mention of a block design, please add more detail to this section to understand why a t-test was needed vs a more appropriate post-hoc test vs control, what the test was to ensure there was no block effect, and if there was a block effect, how was this handled?
11. Please pay special attention when re-writing the results section. Line 189 states there were no changes in body weight, except.... the effects were there, so this opener should be deleted. line 192 states a significant increase in the

0.4 dose group that is not mentioned in the abstract or your data table. line 195 states that "livers of treated mice were enlarged and pale..." leading the reader to think all treated livers are large and pale which you go on to say is not the case. line 200 - be clear that one animal per group is what is shown in Figure S1. line 204 - you say all treatment groups had an effect - that is also not accurate. Where are the in-depth liver measurements and severity information that was described in the methods section? line 206- do you mean that there were vacuoles containing glycogen? I thought glycogen accumulated in the cytoplasm.
Summary - line 247: also stated effects in 0.4 dose group which are not evident in your figures and tables. line 251: state no effects at 0.04 and 0.4 dose groups.

In table 1, please add starting weights and weight gain per mouse.
I am not sure that the footnotes below table 1 are meant for that table. They are not present in the table and don't make sense except for the p-values. Please correct.

Table 2 title says this is bioaccumulation rates - those are not shown. I suggest changing this to Measurements of PFESA-BP2.... LOQ should be reported somewhere here, and the text says that (line 115) the Nafion BP2 concentrations ranged from 0.002-0.8 g/L and that does not match the data in Table 2.

There is a mistake in Table S1 - Female 0.4 reads 0.3, 0.35, 0.4
Figure S1 - the glycogen accumulation really stands out in panel D and the apoptosis and hepatocellular hypertrophy was not commented on extensively. A more detailed description would be possible if the histopathology was more well-powered.

I look forward to seeing your modified manuscript.
